Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 8-13 and 38-55 are pending in the application. Claims 1-7 and 14-37 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. New claims 38-55 are sought to be added. Claims 8-10 and 12 are sought to be amended. Support for amended claim 8 is found, *inter alia*, in the specification on page 2, lines 15-17 and lines 20-23, and page 20, lines 3-7, and in the Examples. Support for the phrase "enzymatically active" in amended claims 9 and 12 and in new claims 39, 42, 45, 48, 51 and 54 is found on page 3, lines 4-7, wherein the peptides or polypeptides (and fragments, thereof) possess ribonuclease activity, and on page 18, lines 5-7, wherein the DNA polymerase must have enzymatic activity such that it can synthesize a nucleic acid molecule from a nucleic acid template, typically in the 5' to 3' direction. Support for new claim 38 is found, *inter alia*, in the specification on page 13, line 21, to page 14, line 5. Support for new claim 44 is found, *inter alia*, in the specification on page 23, line 15. Support for new claim 50 is found, *inter alia*, in original claim 8 and in the specification in the Examples. Support for new claims 39-43, 45-49 and 51-55 is found, *inter alia*, in original claims 9-13.

Claim 8 has been amended to indicate that the starting material is crude DNA (specific starting material in claim 8 only). Claims 9 and 12 have been amended to clarify that the fragments, variants, derivatives and mutants are enzymatically active. Claim 10 has been amended to correct the original improper Markush group language.

The claims and specification have been amended to add the generic terminology for VENT® DNA polymerase and DEEP VENT™ DNA polymerase. Generic terminology for VENT® DNA polymerase, *i.e.*, *Tli* DNA polymerase (from *Thermococcus litoralis*), is disclosed in the present specification, for example at page 17, line 20. The generic terminology for DEEP VENT™ DNA polymerase, *i.e.*, *Pyrococcus* species GB-D DNA polymerase, is disclosed in U.S. Patent No. 5,512,462 (copy attached), which is incorporated by reference into the present specification on page 18, lines 12-19, and on page 28, line 24, to page 29, line 2.

The specification has also been amended to correct typographical errors.

Since the above amendments to the specification and claims are supported by the originally filed disclosure, the amendments do not add new matter and their entry into the application is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding rejections and that they be withdrawn.

Rejection under 35 U.S.C. § 112, second paragraph

The Examiner rejected claim 12 under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the inventive subject matter. Applicants respectfully traverse the rejection.

The Examiner contended that claim 12 is indefinite for reciting the trademarks "VENT™" and "DEEP VENT™" to describe a species of thermostable DNA polymerase. By the foregoing amendments, the corresponding generic terminology for each of these trademarks has been inserted into claim 12 and is recited in new claims 42, 48 and 54. The generic terminology, as discussed above, is fully supported in the specification as originally

filed. Hence, this portion of the rejection under 35 U.S.C. § 112, second paragraph has been rendered moot and reconsideration and withdrawal of the rejection are respectfully requested.

Rejections under 35 U.S.C. § 112, first paragraph

The Examiner rejected claims 9 and 12 under 35 U.S.C. § 112, first paragraph, because the specification "does not reasonably provide enablement for the method of claim 8" wherein the peptide or polypeptide with ribonuclease activity is a fragment, variant, derivative or mutant of the RNases listed in claim 9 (Office action, page 3). The Examiner stated that claim 9 is enabling for the method of claim 8 wherein the peptide or polypeptide having ribonuclease activity is RNase A, RNase T1, RNase H, RNase S, RNase B, RNase C, RNase T2 or an enzymatically active fragment thereof. Similarly, the Examiner stated that the specification "does not reasonably provide enablement for the method of claim 11" wherein the thermostable DNA polymerase is a fragment, variant, derivative or mutant of the thermostable DNA polymerases listed in claim 12 (Office action, pages 3-4). The Examiner stated that claim 12 is enabling for the method of claim 11 wherein the thermostable DNA polymerase is *Taq*, *Tne*, *Tma*, *Tth*, *Tli* or VENT™, *Pfu*, DEEP VENT™, *Pwo*, *Bst*, *Bca*, *Tfl* or an enzymatically active fragment thereof. Applicants respectfully traverse the rejection.

Based on the Examiner's original statement of the rejection, it appears that the Examiner is concerned with the recitation of "fragments, variants, derivatives or mutants" in claims 9 and 12. The Examiner asserted that fragments, variants, derivatives and mutants are not enabled due to the resultant alleged breath of claims 9 and 12. However, it is noted that the Examiner indicated that enzymatically active fragments of the recited peptides or

polypeptides with ribonuclease activity and of the recited DNA polymerases are enabled. To expedite prosecution and without acquiescing in the propriety of the rejection, the claims have been amended to recite that the fragments, variants, derivatives and mutants of the peptides or polypeptides having ribonuclease activity and of the DNA polymerases are enzymatically active. This amendment does not limit the possible peptides and polypeptides having ribonuclease activity and DNA polymerase activity as generically set forth in claim 8 and new claims 38, 44 and 50.

The Examiner further stated that:

> [c]laims 9 and 12 are so broad as to encompass any method of claim 8 wherein said method comprises using any of fragments, variants, derivatives or mutants of those RNases and thermostable DNA polymerases listed in claims 9 and 12 respectively. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of ribonuclease and thermostable DNA polymerase enzymes broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the described method using known or naturally occurring ribonuclease and thermostable DNA polymerase proteins.

Office action, page 4.

Based on this subsequent explanation of the rejection, it appears that the Examiner is concerned with amino acid sequences of proteins and changes within such sequences wherein the proteins and changes thereto are not explicitly set forth in the specification. However, to practice the *claimed* method, one of ordinary skill in the art can take a peptide

or polypeptide, determine whether it has ribonuclease activity, for example, and if it does, use it in the claimed method of nucleic acid synthesis. There is no need for Applicants to establish: (a) regions of protein structure which may be modified without effecting activity; (b) the general tolerance and the extent thereof of ribonucleases and thermostable DNA polymerases to modification; or (c) a rational and predictable scheme for modifying amino acid residues wherein the desired biological function is obtained as asserted by the Examiner on page 5 of the Office action. Applicants assert that any amino acid sequence modification(s) need only result in a peptide or polypeptide with either ribonuclease or DNA polymerase activity. Such activities can be readily determined using standard assays well-within the purview of the skilled artisan. Applicants are not required to set forth something as basic as how to test whether or not a peptide or polypeptide has ribonuclease or DNA polymerase activity.

Regarding Applicants' requirement to set forth guidance as to which peptide or polypeptide is likely to be successful, Applicants have done so. As indicated above, if the peptide or polypeptide has ribonuclease or DNA polymerase activity, then it is expected to be useful in the claimed method.

The Examiner stated that although mutagenesis and recombinant techniques are known, "it is <u>not</u> routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable" (emphasis in the original) (Office action, page 5). The Examiner further stated that the skilled artisan "would expect any tolerance to modification

for a given protein to diminish with each further and additional modification, e.g. multiple substitutions" (Office action, page 5).

As noted above, routine screening for ribonuclease and DNA polymerase activities is well-within the purview of the skilled artisan. Detection of such activities occurs if such activities are present irrespective of the number of modifications in the amino acid sequence of the peptide or polypeptide. The rejection appears to have been written from the viewpoint of an examiner examining claims directed to a newly discovered compound wherein the activity of the compound is known for a particular sequence only or to modified enzymes *per se*. That is not the situation in this case. Applicants are claiming a method. The method employs known enzymes with known activities. Any peptide or polypeptide with the desired enzymatic activities can be employed and determining such well-known activities is routine in the art.

In his conclusion, the Examiner stated that:

> applicants have <u>not</u> provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims drawn to methods of use of those proteins broadly including any number of amino acid modifications of any ribonuclease and thermostable DNA polymerase. The scope of the claims must bear a reasonable correlation with the scope of enablement. Without sufficient guidance, determination of those molecules having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue.

Office action, pages 5-6 (citation omitted).

Again, determining which peptides and polypeptides have the "desired biological characteristics" of ribonuclease or DNA polymerase activity is routine. The particular amino acid sequences of such peptides and polypeptides are not the subject of this invention.

Since the specification provides sufficient guidance enabling one of ordinary skill to make and use the invention as claimed, Applicants assert that the rejection is in error and withdrawal thereof is respectfully requested.

The Examiner rejected claims 9 and 12 under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention" (Office action, page 6). Applicants respectfully traverse the rejection.

Specifically, the Examiner stated that:

> [t]he specification fails to describe in any fashion the physical and/or chemical properties of the claimed genus of proteins necessary for use in the claimed methods and identifies only those ribonucleases and DNA polymerases listed in claims 9 and 12, respectively as a member of the genus having the necessary functional properties. Moreover, the specification fails to describe any other representative species of ribonuclease or thermostable DNA polymerase by any identifying characteristics or properties other than by function. Given this lack of description of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Office action, pages 6-7.

The basis for this rejection is not clear. The Examiner rejected claims 9 and 12, yet indicated that the ribonucleases and DNA polymerases of claims 9 and 12, respectively, are members of the genera of ribonucleases and DNA polymerases required to practice the claimed invention which suggests that the Examiner believes that claims 9 and 12 are supported under 35 U.S.C. § 112, first paragraph. If the Examiner's position is based on the language "fragments, variants, derivatives or mutants thereof," then the rejection is moot

as the intended fragments, variants, derivatives and mutants have been clarified as those that are enzymatically active.

The adequate written description requirement serves to ensure that the inventor had possession, as of the filing date, of the claimed subject matter. However, "how the specification accomplishes this is not material." *In re Wertheim*, 191 U.S.P.Q. 90, 96 (C.C.P.A. 1976). According to the Written Description Guidelines set forth in the Federal Register, vol. 66, No. 4 (January 5, 2001), a "claimed invention as a whole may not be adequately described if the claims require an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art" (page 1105, col. 1). In the current application, the essential features, e.g., ribonuclease and DNA polymerase, are adequately described, conventional in the art and known to one of ordinary skill in the art.

The claims are directed to methods of nucleic acid synthesis and not to a new class of compounds as suggested by the Examiner's explanation of the rejection. The claimed methods employ ribonucleases and DNA polymerases. Applicants have set forth the main shared characteristic of the members of the genera of ribonucleases and DNA polymerases. For example, to be a member of the genus of ribonucleases or DNA polymerases, the peptide or polypeptide employed must have ribonuclease or DNA polymerase activity, respectively. The Examiner is of the position that the recitation of the desired functional property is insufficient. These enzymes are basic tools used by the skilled artisan and therefore, the skilled artisan would readily understand what peptides or polypeptides to employ in the claimed method to obtain ribonuclease activity and DNA polymerase activity. Further, Applicants have provided the skilled artisan with numerous examples of specific

ribonucleases and DNA polymerases that one could employ in the claimed methods. No more is required of Applicants.

Applicants' position is supported by the Written Description Guidelines wherein, regarding an original genus claim, it is stated that the written description requirement may be is satisfied by the following: (1) "sufficient description of a representative number of species by actual reduction to practice" or by (2) disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties (page 1106, col. 3). A description of a representative number of species "does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces." *Id. See also In re Alton*, 37 U.S.P.Q. 2d 1578, 1584 (C.A.F.C. 1996) (stating that, "[i]f a person of ordinary skill in the art would have understood the inventor to have been in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate written description requirement is met.").

Applicants assert that the rejection is in error and withdrawal thereof is respectfully requested.

Rejections under 35 U.S.C. § 102

The Examiner rejected claims 8-12 under 35 U.S.C. § 102(a) as being anticipated by Maudru *et al.* (*Journal of Virological Methods 66:* 247-261 (July 1997)). Applicants respectfully traverse the rejection.

The Examiner stated that Maudru *et al.* anticipate the claimed method since they:

> examine the cause and teach a method for the elimination of background signals in a modified polymerase chain reaction-based reverse transcriptase assay. Maudru et al. teach that

> the background signal of the PCR-based reverse transcriptase (PBRT) assay was due to an intrinsic RNA-dependent DNA polymerase activity of the Taq DNA polymerase enzyme used for the assay. They further teach that this background signal could be eliminated by inserting a ribonuclease digestion step prior to amplifying the cDNA product of the RT reaction by PCR.

Office action, page 7.

Under 35 U.S.C. § 102, a claim can only be anticipated if every element in the claim is expressly or inherently disclosed in a single prior art reference. *See Kalman v. Kimberly Clark Corp.*, 713 F.2d 760, 771 (Fed. Cir. 1983), *cert. denied*, 465 U.S. 1026 (1984). Moreover, "[t]o anticipate a claim, a reference must disclose every element of the challenged claim and enable one skilled in the art to make the anticipating subject matter." *PPG Industries, Inc. v. Guardian Industries Corp.*, 75 F.3d 1558, 1566 (Fed. Cir. 1996); *see also In re Donohue*, 766 F.2d 531, 533 (Fed. Cir. 1985).

Amended claim 8 requires a crude DNA-containing preparation as the starting sample. Maudru *et al.* require purified RNA as the starting material. Thus, claims 8 and claims 9-12 (dependent thereon) cannot be anticipated by this reference.

New claims 38-43 exclude RNase H as the peptide or polypeptide having ribonuclease activity. The Maudru *et al.* method relies on the action of RNase H. Thus, claims 38-43 cannot be anticipated by Maudru *et al.*

New claims 44-49 exclude cDNA as the nucleic acid template. In the Maudru *et al.* method, nucleic acid synthesis first occurs during the reverse transcriptase step resulting in an RNA/cDNA hybrid. The cDNA is then used as the nucleic acid template during the PCR process. Since the Maudru *et al.* method employs cDNA as the nucleic acid template during the nucleic acid synthesis in PCR, claims 44-49 cannot be anticipated by Maudru *et al.*

New claims 50-55 require a specific sequence of steps wherein the peptide or polypeptide with ribonuclease activity is added *prior* to nucleic acid synthesis. In the Maudru *et al.* method, nucleic acid synthesis begins during the reverse transcriptase step wherein the RNA is subjected to a mixture containing buffer, dNTPs, DTT, oligodeoxynucleotide RT primer and RNasin. At this point, the RNA serves as the template to create an RNA/cDNA hybrid. This hybrid is then subjected to PCR. Thus, prior to the addition of the RNase and the DNA polymerase, the nucleic acid synthesis has already occurred. Thus, claims 50-55 cannot be anticipated by Maudru *et al.* and withdrawal of the rejection is respectfully requested.

The Examiner rejected claims 8-12 under 35 U.S.C. § 102(b) as being anticipated by Don *et al.* (*Nucleic Acids Research 21(3):* 783 (1993)). Applicants respectfully traverse the rejection.

The Examiner stated that Don *et al.* anticipate the claimed method because they teach a "one tube reaction" for synthesis and amplification of total cDNA from a small number of cells as well as a method for synthesizing nucleic acid by mixing a template, reverse transcriptase, RNase H, T4 DNA polymerase and *Taq* DNA polymerase.

Under 35 U.S.C. § 102, a claim can only be anticipated if every element in the claim is expressly or inherently disclosed in a single prior art reference. *See Kalman v. Kimberly Clark Corp.*, 713 F.2d 760, 771 (Fed. Cir. 1983), *cert. denied*, 465 U.S. 1026 (1984). Moreover, "[t]o anticipate a claim, a reference must disclose every element of the challenged claim and enable one skilled in the art to make the anticipating subject matter." *PPG Industries, Inc. v. Guardian Industries Corp.*, 75 F.3d 1558, 1566 (Fed. Cir. 1996); *see also In re Donohue*, 766 F.2d 531, 533 (Fed. Cir. 1985).

Amended claim 8 requires a crude DNA-containing preparation as the starting sample. Don *et al.* require purified RNA as the starting material. Thus, claims 8 and claims 9-12 (dependent thereon) cannot be anticipated by this reference.

New claims 38-43 exclude RNase H as the peptide or polypeptide having ribonuclease activity. The Don *et al.* method relies on the action of RNase H. Thus, claims 38-43 cannot be anticipated by Don *et al.*

New claims 44-49 exclude cDNA as the nucleic acid template. In the Don *et al.* method, nucleic acid synthesis first occurs during the reverse transcriptase step resulting in an RNA/cDNA hybrid. The cDNA is then used as the nucleic acid template during the PCR process. Since the Don *et al.* method employs cDNA as the nucleic acid template during the nucleic acid synthesis in PCR, claims 44-49 cannot be anticipated by Don *et al.*

New claims 50-55 require a specific sequence of steps wherein the peptide or polypeptide with ribonuclease activity is added *prior* to nucleic acid synthesis. In the Don *et al.* method, nucleic acid synthesis begins during the reverse transcriptase step wherein the RNA is subjected to a mixture containing buffer, dNTPs, DTT, oligodeoxynucleotide RT primer and RNasin. At this point, the RNA serves as the template to create an RNA/cDNA hybrid. This hybrid is then subjected to PCR. Thus, prior to the addition of the RNase and the DNA polymerase, the nucleic acid synthesis has already occurred. Thus, claims 50-55 cannot be anticipated by Don *et al.* and withdrawal of the rejection is respectfully requested.

Rejection under 35 U.S.C. § 103

The Examiner rejected claim 13 under 35 U.S.C. § 103(a) as being unpatentable over Maudru *et al.* (*Journal of Virological Methods 66:* 247-261 (July 1997)) or Don *et al.* (*Nucleic Acids Research 21(3):* 783 (1993)). Applicants respectfully traverse the rejection.

The Examiner repeated the alleged teachings of Maudru *et al.* and Don *et al.* and stated that:

> [o]ne of ordinary skill in the art would have been motivated to use the method of claim 10 to synthesize a nucleic acid molecule wherein one or more of said nucleotides are detectably labeled so that the synthesized DNA molecule could be used as a probe to isolate similar DNA molecules from a DNA library, or so that the label could be used as a means of measuring the amount of DNA synthesized. One would have had a reasonable expectation of success based on the knowledge well known in the art of using radioactive nucleotides in DNA synthesis reactions to detectably label the synthesized product.

Office action, page 9.

Applicants have amended claims 8-12 (claim 13 is dependent upon claim 10 which is dependent upon independent claim 8) and have overcome the rejections under 35 U.S.C. §§ 102(a) and 102(b) as indicated above. Since claims 8-12 are free of the prior art, claim 13, dependent thereon, must also be free of the prior art. Thus, the rejection under 35 U.S.C. § 103(a) is moot and withdrawal thereof is respectfully requested.

Other Matters

Applicants request that the Examiner initial and date the considered references on the second page of the Information Disclosure Statement filed September 1, 1999, and forward a copy of that second page to Applicants with the next Office action.

Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Lawrence B. Burgovsky
Reg # 35,086
for

Kristin K. Vidovich
Attorney for Applicants
Registration No. 41,448

Date: April 2, 2007

1100 New York Avenue, N.W.
Suite 600
Washington, D.C. 20005-3934
(202) 371-2600

Version with markings to show changes made

Specification:

Substitute paragraph bridging pages 3-4:

Another aspect of the invention provides a method for synthesizing nucleic acids, specifically DNA, using one or more enzymes, proteins or peptides (or fragments, mutants, derivatives or variants thereof) possessing RNase activity. Preferably, the ribonucleases used substantially lack DNase activity, and more preferably lack detectable levels of DNase activity. The method provided for synthesizing DNA (or other polynucleotides) comprises the step of mixing one or more desired templates with one or more [enzyme] enzymes, proteins or peptides (or fragments, mutants, derivatives or variants thereof) possessing RNase activity along with other reagents required for polynucleotide synthesis. Reagents required for polynucleotide synthesis include one or more nucleotides (e.g. dNTPs) or derivatives thereof, one or more polynucleotide primers, one or more DNA polymerases, and the like. The invention thus relates to a method of synthesizing a nucleic acid molecule comprising: (a) mixing a nucleic acid template with one or more DNA polymerases and with one or more RNases of the invention; and (b) incubating said mixture under conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of said template. Thus, ribonuclease treatment may be conducted simultaneously with the nucleotide synthesis reaction and thus one or more ribonucleases may be added in conjunction with other components necessary for a nucleotide synthesis (e.g. nucleotides, primers, one or more DNA polymerases and the like). In a related aspect, one or more ribonucleases may be added to a sample prior to the nucleic acid synthesis step. Thus, a sample may be treated in accordance with the invention with one or more ribonucleases and following such treatment, nucleic acid synthesis in the presence of one or more polymerases may be conducted. In this aspect, the ribonuclease activity may or may not be inactivated after treatment but before synthesis by well known techniques. Thus, ribonuclease treatment may be accomplished prior to and/or during the nucleic acid synthesis reaction.

Substitute paragraph bridging pages 4-5:

Another aspect of the invention relates to amplification of nucleic acid molecules, for example a polymerase chain reaction or in an application of PCR, using one or more ribonucleases in accordance with the invention. The invention thus relates to a method for amplifying a double stranded DNA molecule, comprising: (a) providing a first and second primer, wherein said first primer is complementary to a sequence at or near the 3'-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence at or near the 3'-termini of the second strand of said DNA molecule; (b) hybridizing said first primer to said first strand and said second primer to said second strand in the presence of one or more DNA polymerases and one or more RNases of the invention, under conditions such that a third DNA molecule complementary to said first strand and a fourth DNA molecule complementary to said second strand are synthesized; (c) denaturing said first and third strand, and said second and fourth strands; and (d) repeating steps (a) to (c) one or more times. For amplification of nucleic acid molecules, ribonuclease treatment may also be performed prior to and/or during nucleic acid synthesis or amplification. Thus, according to the invention, ribonucleases may be used at any step and may be removed or inactivated at any step. Removal or inactivation of ribonucleases can be accomplished using techniques well known to those [in] <u>of</u> ordinary skill in the art (e.g. chemical extraction (phenol and/or [chlorophorm] <u>chloroform</u>), precipitation, protein denaturation, heat, etc.).

Substitute paragraph one on page 13:

Prior to the inventors' work, polynucleotide synthesis *in vitro* was performed without RNase. In a variety of nucleic acid synthesis procedures, the subject compositions provide superior synthesis results, as compared with synthesis results obtained without RNase. The composition is especially useful in DNA synthesis when the sample is crude, i.e. prepared rapidly such that it contains contaminating RNA. In such situations, the [results] <u>result</u> achieved, i.e., the amount of synthesis product produced, [are] <u>is</u> significantly greater than the amount of synthesis product obtained without RNase. Other advantages of the subject

compositions and methods include increased product length, as well as the synthesis of polynucleotides that could not be synthesized previously, i.e., in the absence of RNase.

Substitute paragraph two on page 13:

The subject invention thus provides novel compositions for use in synthesizing nucleic acids, particularly DNA. The subject compositions comprise one or more ribonucleases and may optimally further [comprises] comprise one or more DNA polymerases. Such [composition] compositions may also comprise one or more components selected from the group consisting of one or more nucleotides, one or more primers, one or more buffers suitable for nucleic acid synthesis and/or one or more templates.

Substitute paragraph bridging pages 13-14:

Enzymes, [protein] proteins or [peptide] peptides (or [fragment] fragments, [mutant] mutants, [variant] variants or derivatives thereof) possessing RNase activity for use in the present compositions and methods may be isolated from natural sources, produced through recombinant DNA techniques, or chemically synthesized. Such enzymes that possess RNase activity and their properties are detailed in The Enzymes, Vol. IV (P.D. Boyer, ed.) Academic Press, San Diego. Examples of enzymes that possess RNase activity useful in the compositions and methods of the present invention include RNase A, RNase H, RNase T1, RNase T2, RNase S, RNase B, RNase C or variants, derivatives, fragments or mutants thereof and the like.

Substitute paragraph one on page 14:

RNase A, a preferred enzyme for use in the present invention, is an endoribonuclease from bovine pancreas that hydrolyzes RNA after C (cytosine) and U (Uracil) residues [[] (Richard and Wyckoff (1971) [*In*] The Enzymes, Vol. IV (P.D. Boyer, ed.) pp. 647-806. Academic Press, San Diego[]]). Cleavage occurs between the 3'-phosphate group of a

pyrimidine ribonucleotide and the 5'-hydroxyl of the adjacent nucleotide. The reaction generates a 2':3' cyclic phosphate which then is hydrolyzed to the corresponding 3'-nucleoside [phosphates] phosphate.

Substitute paragraph two on page 14:

Ribonuclease T1 from *Aspergillus oryzae* is an endoribonuclease that hydrolyzes RNA after G residues [[] (Uchida and Egami (1971) [*In:*] The Enzymes, [Vol] Vol. IV (P.D. Boyer, [Ed.] ed.) pp. 205-250. Academic Press, San Diego[]]). Cleavage occurs between the 3'-phosphate group of a guanine ribonucleotide and the 5'-hydroxyl of the adjacent nucleotide. The reaction generates a 2':3' cyclic phosphate which then is hydrolyzed to the corresponding 3'-nucleoside [phosphates] phosphate.

Substitute paragraph three on page 15:

RNase H from *E. coli* is an endoribonuclease that specifically hydrolyzes the phosphodiester bonds of RNA in RNA:DNA duplexes to generate products with 3'hydroxyl and 5'phosphate ends [[] (Berkower *et al.* (1973) *J. Biol. Chem.* 248:5914-5924[]]).

Substitute paragraph bridging pages 17-18:

A variety of polypeptides having polymerase activity are useful in accordance with the present invention. Included among these polypeptides are enzymes such as nucleic acid polymerases (including DNA polymerases). Such polymerases include, but are not limited to, *Thermus thermophilus (Tth)* DNA polymerase, *Thermus aquaticus (Taq)* DNA polymerase, *Thermotoga neopolitana (Tne)* DNA polymerase, *Thermotoga maritima (Tma)* DNA polymerase, *Thermococcus litoralis* [(*Tli* or VENT™)] (*Tli* or VENT®) DNA polymerase, *Pyrococcus furiosus (Pfu)* DNA polymerase, [DEEPVENT™] *Pyrococcus* species GB-D (DEEP VENT™) DNA polymerase, *Pyrococcus woosii (Pwo)* DNA polymerase, *Bacillus sterothermophilus (Bst)* DNA polymerase, *Bacillus caldophilus (Bca)*

DNA polymerase, *Sulfolobus acidocaldarius (Sac)* DNA polymerase, *Thermoplasma acidophilum (Tac)* DNA polymerase, *Thermus flavus (Tfl/Tub)* DNA polymerase, *Thermus ruber (Tru)* DNA polymerase, *Thermus brockianus* (DYNAZYME™) DNA polymerase, *Methanobacterium thermoautotrophicum (Mth)* DNA polymerase, mycobacterium DNA polymerase *(Mtb, Mlep)*, and mutants, [and] variants and derivatives thereof.

Substitute paragraph bridging pages 18-19:

Polymerases used in accordance with the invention may be any enzyme that can synthesize a nucleic acid molecule from a nucleic acid template, typically in the 5' to 3' direction. The nucleic acid polymerases used in the present invention may be mesophilic or thermophilic, and are preferably thermophilic. Preferred mesophilic DNA polymerases include T7 DNA polymerase, T5 DNA polymerase, Klenow fragment DNA polymerase, DNA polymerase III and the like. Preferred thermostable DNA polymerases that may be used in the methods of the invention include *Taq*, *Tne*, *Tma*, *Pfu*, *Tfl*, *Tth*, Stoffel fragment, *Tli* (VENT®) [VENT™] and [DEEPVENT™] *Pyrococcus* species GB-D (DEEP VENT™) DNA polymerases, and mutants, variants and derivatives thereof (U.S. Patent No. 5,436,149; U.S. Patent No. 4,889,818; U.S. Patent No. 4,965,188; U.S. Patent No. 5,079,352; U.S. Patent No. 5,614,365; U.S. Patent No. 5,374,553; U.S. Patent No. 5,270,179; U.S. Patent No. 5,047,342; U.S. Patent No. 5,512,462; WO 92/06188; WO 92/06200; WO 96/10640; Barnes, W.M., *Gene 112*:29-35 (1992); Lawyer, F.C., *et al., PCR Meth. Appl. 2*:275-287 (1993); Flaman, J.-M, *et al., Nucl. Acids Res. 22*(15):3259-3260 (1994)). For amplification of long nucleic acid molecules (e.g., nucleic acid molecules longer than about 3-5 Kb in length), at least two DNA polymerases (one substantially lacking 3' exonuclease activity and the other having 3' exonuclease activity) are typically used. See U.S. Patent No. 5,436,149; U.S. Patent No. 5,512,462; Barnes, W.M., *Gene 112*:29-35 (1992), the disclosures of which are incorporated herein in their entireties. Examples of DNA polymerases substantially lacking in 3' exonuclease activity include, but are not limited to, *Taq, Tne*(exo$^-$), *Tma*(exo$^-$), *Pfu*(exo$^-$), *Pwo*(exo$^-$) and *Tth* DNA polymerases, and mutants, variants and derivatives thereof.

Substitute paragraph bridging pages 21-22:

When sequencing a DNA molecule, ddNTPs lack a hydroxyl residue at the 3' position of the deoxyribose base and thus, although they can be incorporated by DNA polymerases into the growing DNA chain, the absence of the 3'-hydroxy residue prevents formation of the next phosphodiester bond resulting in termination of [extention] extension of the DNA molecule. Thus, when a small amount of one ddNTP is included in a sequencing reaction mixture, there is competition between extension of the chain and base-specific termination resulting in a population of synthesized DNA molecules which are shorter in length than the DNA template to be sequenced. By using four different ddNTPs in four separate enzymatic reactions, populations of the synthesized DNA molecules can be separated by size so that at least a part of the nucleotide sequence of the original DNA molecule can be determined. DNA sequencing by dideoxy-nucleotides is well known and is described by Sambrook *et al.*, [In:] *Molecular Cloning, a Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). As will be readily recognized, the polymerases of the present invention may be used in such sequencing reactions.

Substitute paragraph one on page 23:

The invention also relates to amplification or synthesis of cDNA. As is known, cDNA is prepared from mRNA templates. See U.S. Patent Nos. 5,405,776 and 5,244,797. The double stranded cDNA is typically cloned into a host cell and such host cells may be used in the present invention.

Substitute paragraph bridging pages 24-25:

A kit for sequencing may comprise a number of container means. A first container means may, for example, comprise one or more RNases of the invention. A second container means may comprise a polymerase [of] or combination of polymerases. A third container may comprise one or a number of types of nucleotides needed to synthesize a

DNA molecule complementary to a DNA template. A fourth container means may comprise one or more or a number of different types of terminators (such as dideoxynucleoside triphosphates). A fifth container means may comprise pyrophosphatase. In addition to the above container means, additional container means may be included in the kit which comprise one or a number of primers and/or a suitable sequencing buffer.

Substitute paragraph one after "EXAMPLE 1:"

Samples from a cDNA library which could not be amplified using a reaction buffer without RNase were grown in I ml of LB (100 ug [amplicillin] ampicillin/ml) overnight at 30°C. Also, 5 ul from each of the fresh cultures were dotted on an ampicillin plate and grown overnight at 30°C.

Claims:

8. (once amended) A method for synthesizing a nucleic acid molecule from a crude preparation containing DNA, said method comprising:

a) mixing the crude preparation containing DNA wherein the DNA functions as a nucleic acid template, with one or more DNA polymerases, and one or more peptides or polypeptides having ribonuclease activity; and

b) incubating said mixture under [condition] conditions sufficient to synthesize a nucleic acid molecule complementary to all or a portion of said template.

9. (once amended) The method according to claim 8, wherein said peptide or polypeptide having ribonuclease activity is selected from the group consisting of: RNase A, RNase T1, RNase H, RNase S, RNase B, RNase C, [and] RNase T2 [or] and enzymatically active fragments, variants, derivatives or mutants thereof.

10. (once amended) The method according to claim 8, wherein said mixture further [comprising] comprises one or more components selected from the group consisting of: a) [one or more nucleotides] at least one nucleotide; b) [one or more DNA polymerases; c) one or more] at least one suitable [buffers] buffer for nucleic acid synthesis; and [d) one or more primers] c) at least one primer.

12. (once amended) The method according to claim 11, wherein said thermostable DNA polymerase is selected from the group consisting of: *Taq* DNA polymerase, *Tne* DNA polymerase, *Tma* DNA polymerase, *Tth* DNA polymerase, *Tli* [or VENT™] DNA polymerase, *Pfu* DNA polymerase, [DEEPVENT™] *Pyrococcus* species GB-D DNA polymerase, *Pwo* DNA polymerase, *Bst* DNA polymerase, *Bca* DNA polymerase, [and] *Tfl* DNA polymerase [or] and enzymatically active fragments, variants, derivatives or mutants thereof.